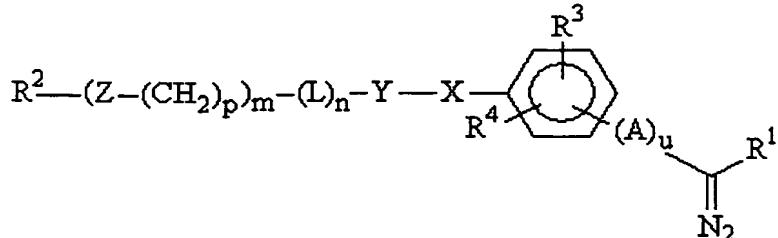


## **CLAIMS**

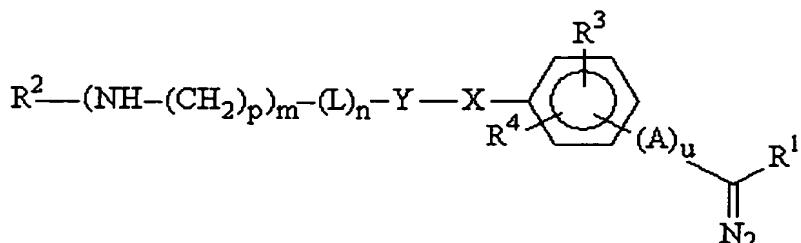
## 1. A temperature-stable labeling reagent of formula (0):



## 5 in which:

- R<sup>1</sup> represents H or an alkyl, aryl or substituted aryl group,
  - R<sup>2</sup> represents a detectable marker or at least two detectable markers interlinked by at least one multimeric structure,
  - L is a linker arm comprising a linear chain of at least two covalent bonds and n is an integer equal to 0 or 1,
  - R<sup>3</sup> and R<sup>4</sup> represent, independently of one another: H, NO<sub>2</sub>, Cl, Br, F, I, R<sup>2</sup> -(L)<sub>n</sub>-Y-X-, OR, SR, NR<sub>2</sub>, R, NHCOR, CONHR, COOR, -CO-NH-(CH<sub>2</sub>)<sub>3</sub>-(O-CH<sub>2</sub>-CH<sub>2</sub>)<sub>3</sub>-CH<sub>2</sub>-NH-R<sup>2</sup>, -CO-NH-(CH<sub>2</sub>)<sub>3</sub>-(O-CH<sub>2</sub>-CH<sub>2</sub>)<sub>4</sub>-CH<sub>2</sub>-NH-R<sup>2</sup> with R = alkyl or aryl,
  - A is a linker arm comprising at least one covalent double bond enabling the conjugation of the diazo function with the aromatic ring and u is an integer between 0 and 2, preferably 0 or 1,
  - -Y-X- represents -CONH-, -NHCO-, -CH<sub>2</sub>O-, -CH<sub>2</sub>S-,
  - -Z- represents -NH-, -NHCO-, -CONH- or -O-,
  - m is an integer between 1 and 10, preferably between 1 and 3, and
  - p is an integer between 1 and 10, preferably between 1 and 3.

2. The labeling reagent, as claimed in claim 1, of formula (1):



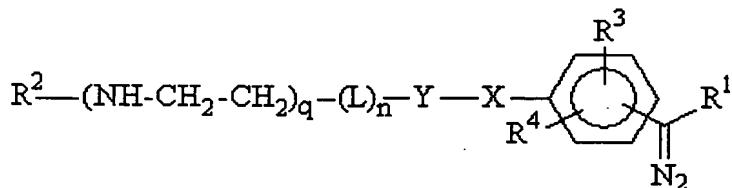
in which :

- R<sup>1</sup> represents H or an alkyl, aryl or substituted aryl group,
- R<sup>2</sup> represents a detectable label or at least two detectable labels interlinked by at least one multimeric structure,
- 5 • L is a linker arm comprising a linear chain of at least two covalent bonds and n is an integer equal to 0 or 1,
- R<sup>3</sup> and R<sup>4</sup> represent, independently of one another: H, NO<sub>2</sub>, Cl, Br, F, I, R<sup>2</sup> -(L)<sub>n</sub>-Y-X-, OR, SR, NR<sub>2</sub>, R, NHCOR, CONHR, COOR, -CO-NH-(CH<sub>2</sub>)<sub>3</sub>-(O-CH<sub>2</sub>-CH<sub>2</sub>)<sub>3</sub>-CH<sub>2</sub>-NH-R<sup>2</sup>, -CO-NH-(CH<sub>2</sub>)<sub>3</sub>-(O-CH<sub>2</sub>-CH<sub>2</sub>)<sub>4</sub>-CH<sub>2</sub>-NH-R<sup>2</sup> with R = alkyl or aryl, and
- 10 • -Y-X- represents -CONH-, -NHCO-, -CH<sub>2</sub>O-, -CH<sub>2</sub>S-,
- m is an integer between 1 and 10, preferably between 1 and 3, and
- p is an integer between 1 and 10, preferably between 1 and 3.

3. The reagent as claimed in either one of claims 1 and 2, characterized in that p is

15 less than or equal to m.

4. The reagent as claimed in one of claims 1 to 3, of formula (2):

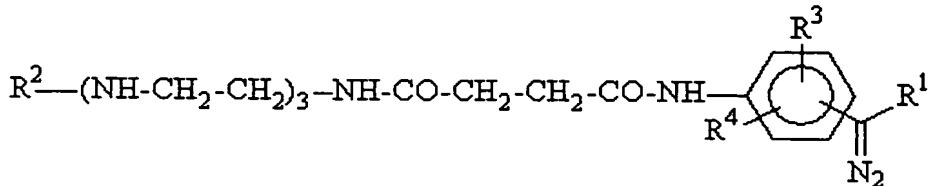


in which:

- 20 • R<sup>1</sup> represents H or an alkyl, aryl or substituted aryl group,
- R<sup>2</sup> represents a detectable label or at least two detectable labels interlinked by means of at least one multimeric structure,
- L is a linker arm comprising a linear chain of at least two covalent bonds and n is an integer equal to 0 or 1,
- 25 • R<sup>3</sup> and R<sup>4</sup> represent, independently of one another: H, NO<sub>2</sub>, Cl, Br, F, I, R<sup>2</sup> -(L)<sub>n</sub>-Y-X-, OR, SR, NR<sub>2</sub>, R, NHCOR, CONHR, COOR, -CO-NH-(CH<sub>2</sub>)<sub>3</sub>-(O-CH<sub>2</sub>-CH<sub>2</sub>)<sub>3</sub>-CH<sub>2</sub>-NH-R<sup>2</sup>, -CO-NH-(CH<sub>2</sub>)<sub>3</sub>-(O-CH<sub>2</sub>-CH<sub>2</sub>)<sub>4</sub>-CH<sub>2</sub>-NH-R<sup>2</sup> with R = alkyl or aryl, and

- q is an integer between 1 and 10, preferably between 1 and 3.

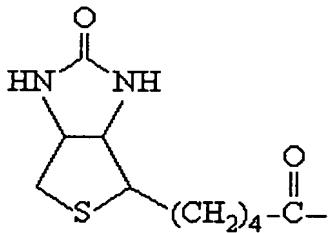
5. The reagent, as claimed in any one of claims 1 to 4, of formula (3):



5 in which:

- R<sup>1</sup> represents H or an alkyl, aryl or substituted aryl group,
- R<sup>2</sup> represents a detectable label or at least two detectable labels interlinked by means of at least one multimeric structure,
- L is a linker arm comprising a linear chain of at least two covalent bonds and n is an integer equal to 0 or 1, and
- R<sup>3</sup> and R<sup>4</sup> represent, independently of one another: H, NO<sub>2</sub>, Cl, Br, F, I, R<sup>2</sup> -(L)<sub>n</sub>-Y-X-, OR, SR, NR<sub>2</sub>, R, NHCOR, CONHR, COOR, -CO-NH-(CH<sub>2</sub>)<sub>3</sub>-(O-CH<sub>2</sub>-CH<sub>2</sub>)<sub>3</sub>-CH<sub>2</sub>-NH-R<sup>2</sup>, -CO-NH-(CH<sub>2</sub>)<sub>3</sub>-(O-CH<sub>2</sub>-CH<sub>2</sub>)<sub>4</sub>-CH<sub>2</sub>-NH-R<sup>2</sup> with R = alkyl or aryl.

15 6. The reagent as claimed in claim 5, characterized in that R<sup>2</sup> consists of a D-biotin residue of formula (4):



20 7. The reagent as claimed in any one of claims 1 to 6, characterized in that R<sup>1</sup> consists of: CH<sub>3</sub>, and R<sup>3</sup> and R<sup>4</sup> each represent: H.

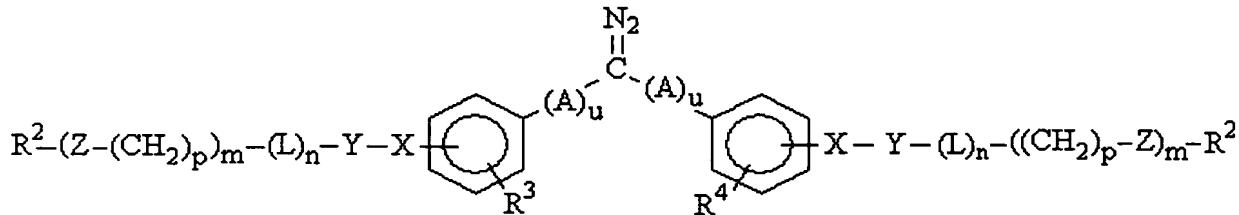
8. The reagent as claimed in any one of claims 1 to 7, in which the structure -(L)<sub>n</sub>- consists of:

- spermine or N,N'-bis(3-aminopropyl)-1,4-diaminobutane: NH<sub>2</sub>-(CH<sub>2</sub>)<sub>3</sub>-NH-(CH<sub>2</sub>)<sub>4</sub>-NH-

$(CH_2)_3-NH_2$ , or

- spermidine or N-(3-aminopropyl)-1,4-butanediamine:  $H_2N-(CH_2)_4-NH-(CH_2)_3-NH_2$ , or
- a derivative containing an alanine motif:  $NH_2-CH_2-CH_2-COOH$ .

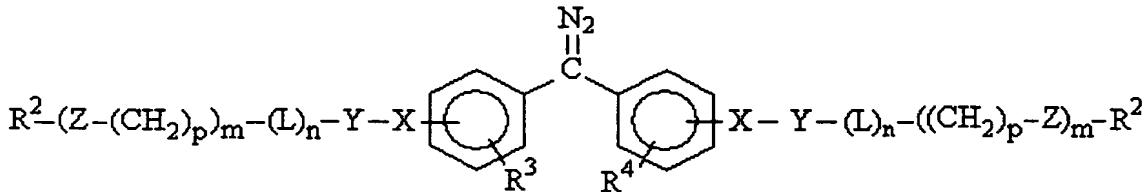
5           9. A temperature-stable labeling reagent of formula (6):



in which:

- $R^1$  represents H or an alkyl, aryl or substituted aryl group,
- $R^2$  represents a detectable label or at least two detectable labels interlinked by at least one multimeric structure,
- L is a linker arm comprising a linear chain of at least two covalent bonds and n is an integer equal to 0 or 1,
- $R^3$  and  $R^4$  represent independently of one another: H,  $NO_2$ , Cl, Br, F, I,  $R^2-(L)_n-Y-X-$ , OR, SR,  $NR_2$ , R,  $NHCOR$ ,  $CONHR$ ,  $COOR$ ,  $-CO-NH-(CH_2)_3-(O-CH_2-CH_2)_3-CH_2-NH-R^2$ ,  $-CO-NH-(CH_2)_3-(O-CH_2-CH_2)_4-CH_2-NH-R^2$  with R = alkyl or aryl,
- A is a linker arm comprising at least one covalent double bond enabling the conjugation of the diazo function with the aromatic ring and u is an integer between 0 and 2, preferably 0 or 1,
- $-Y-X-$  represents  $-CONH-$ ,  $-NHCO-$ ,  $-CH_2O-$ ,  $-CH_2S-$ ,
- $-Z-$  represents  $-NH-$ ,  $-NHCO-$ ,  $-CONH-$  or  $-O-$ ,
- m is an integer between 1 and 10, preferably between 1 and 3, and
- p is an integer between 1 and 10, preferably between 1 and 3.

15           10. The labeling reagent, as claimed in claim 9, of formula (7):



in which:

- $R^1$  represents H or an alkyl, aryl or substituted aryl group,
- $R^2$  represents a detectable label or at least two detectable labels interlinked by at least one multimeric structure,
- L is a linker arm comprising a linear chain of at least two covalent bonds and n is an integer equal to 0 or 1,
- $R^3$  and  $R^4$  represent, independently of one another: H, NO<sub>2</sub>, Cl, Br, F, I,  $R^2-(L)_n-Y-X-$ , OR, SR, NR<sub>2</sub>, R, NHCOR, CONHR, COOR, -CO-NH-(CH<sub>2</sub>)<sub>3</sub>-(O-CH<sub>2</sub>-CH<sub>2</sub>)<sub>3</sub>-CH<sub>2</sub>-NH-R<sup>2</sup>, -CO-NH-(CH<sub>2</sub>)<sub>3</sub>-(O-CH<sub>2</sub>-CH<sub>2</sub>)<sub>4</sub>-CH<sub>2</sub>-NH-R<sup>2</sup> with R = alkyl or aryl,
- -Y-X- represents -CONH-, -NHCO-, -CH<sub>2</sub>O-, -CH<sub>2</sub>S-,
- -Z- represents -NH-, -NHCO-, -CONH- or -O-,
- m is an integer between 1 and 10, preferably between 1 and 3, and
- p is an integer between 1 and 10, preferably between 1 and 3.

15            11. The reagent as claimed in any one of claims 1 to 10, characterized in that L comprises a motif -(O-CH<sub>2</sub>-CH<sub>2</sub>)-, repeated from 1 to 20 times, preferably from 1 to 10 times, and even more preferably from 2 to 5 times, -Z- then being represented by -NH-, -NHCO- or -CONH-.

20            12. A method for the synthesis of a labeling reagent as claimed in any one of claims 1 to 11, comprising the following steps:

- a) a label or a label precursor having a reactive function  $R^6$  is provided,
- b) a linker arm of formula (8):

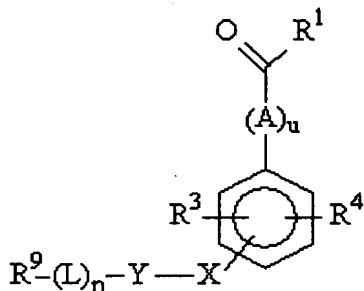


25

is provided,

in which formula:

- -Z- represents -NH-, -NHCO-, -CONH- or -O-,
  - m is an integer between 1 and 10, preferably between 1 and 3,
  - p is an integer between 1 and 10, preferably between 1 and 3,
  - R<sup>7</sup> and R<sup>8</sup> represent two reactive functions which may be identical or different,
- 5           c) the reactive function R<sup>6</sup> of said label or label precursor and the function R<sup>7</sup> of the linker arm of formula (8) are reacted together in the presence of at least one coupling agent so as to form a covalent bond, R<sup>6</sup> and R<sup>7</sup> being complementary,
- d) a derivative of formula (9):



- 10       is provided,
- in which formula:
- R<sup>1</sup> represents H or an alkyl, aryl or substituted aryl group,
  - L is a linker arm comprising a linear chain of at least two covalent bonds and n is an integer equal to 0 or 1,
- 15       • R<sup>3</sup> and R<sup>4</sup> represent, independently of one another: H, NO<sub>2</sub>, Cl, Br, F, I, R<sup>2</sup> -(L)<sub>n</sub>-Y-X-, OR, SR, NR<sub>2</sub>, R, NHCOR, CONHR, COOR, -CO-NH-(CH<sub>2</sub>)<sub>3</sub>-(O-CH<sub>2</sub>-CH<sub>2</sub>)<sub>3</sub>-CH<sub>2</sub>-NH-R<sup>2</sup>, -CO-NH-(CH<sub>2</sub>)<sub>3</sub>-(O-CH<sub>2</sub>-CH<sub>2</sub>)<sub>4</sub>-CH<sub>2</sub>-NH-R<sup>2</sup> with R = alkyl or aryl,
- -Y-X- represents -CONH-, -NHCO-, -CH<sub>2</sub>O-, -CH<sub>2</sub>S-,
  - A is a linker arm comprising at least one covalent double bond enabling the conjugation of the diazomethyl function with the aromatic ring and u is an integer equal to 0 or 1, and
  - R<sup>9</sup> represents a reactive function complementary to R<sup>8</sup>,
- 20           e) the reactive function R<sup>9</sup> of the derivative of formula (9) and the function R<sup>8</sup> of the linker arm of formula (8) are reacted together in the presence of at least one coupling agent so as to form a covalent bond,
- 25           f) the hydrazine or one of its derivatives is reacted with the ketone or aldehyde function so as to form a hydrazone, and

g) the hydrazone is converted to a diazomethyl function by means of an appropriate treatment.

13. The method of synthesis as claimed in claim 12, characterized in that it comprises:

- 5 • an additional step consisting of protection of the ketone or aldehyde function of compound (9), and
- a subsequent additional step consisting of deprotection of said ketone or aldehyde function.

14. A method for the labeling of a biological molecule, in particular a nucleic acid, comprising bringing into contact, in homogeneous solution, in a substantially aqueous buffer, a biological molecule and a reagent, obtained as claimed in any one of claims 1 to 11.

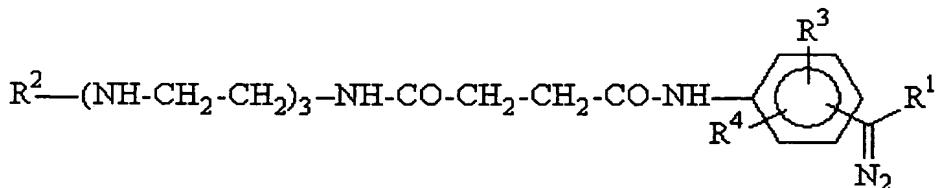
15. A labeled biological molecule which can be obtained by means of the method as claimed in claim 14.

15

16. A method for the labeling and fragmentation of a single-stranded or double-stranded nucleic acid, comprising the following steps:

- fragmenting the nucleic acid,
  - attaching a label to at least one of the fragments by means of a labeling reagent chosen from the reagents obtained as claimed in any one of claims 1 to 11,
- 20 said reagent coupling covalently and predominantly on at least one phosphate of said fragment.

17. The method as claimed in claim 16, characterized in that the labeling reagent is chosen from the compounds of formula (3):



in which :

- R<sup>1</sup> represents H or an alkyl, aryl or substituted aryl group,

- R<sup>2</sup> represents a detectable label or at least two detectable labels interlinked by at least one multimeric structure,
  - L is a linker arm comprising a linear chain of at least two covalent bonds and n is an integer equal to 0 or 1, and
- 5     • R<sup>3</sup> and R<sup>4</sup> represent, independently of one another: H, NO<sub>2</sub>, Cl, Br, F, I, R<sup>2</sup> -(L)<sub>n</sub>-Y-X-, OR, SR, NR<sub>2</sub>, R, NHCOR, CONHR, COOR, -CO-NH-(CH<sub>2</sub>)<sub>3</sub>-(O-CH<sub>2</sub>-CH<sub>2</sub>)<sub>3</sub>-CH<sub>2</sub>-NH-R<sup>2</sup>, -CO-NH-(CH<sub>2</sub>)<sub>3</sub>-(O-CH<sub>2</sub>-CH<sub>2</sub>)<sub>4</sub>-CH<sub>2</sub>-NH-R<sup>2</sup> with R = alkyl or aryl.

18. The method as claimed in either one of claims 16 and 17, characterized in that the  
10 fragmentation and the labeling are carried out in two steps.

19. The method as claimed in either one of claims 16 and 17, characterized in that the  
fragmentation and the labeling are carried out in one step.

15     20. The method as claimed in any one of claims 16 to 17, characterized in that the  
labeling is carried out in a substantially aqueous homogeneous solution.

21. The method as claimed in any one of claims 16 to 20, characterized in that the  
fragmentation is carried out by an enzymatic, physical or chemical process.

20     22. A labeled nucleic acid which can be obtained by means of the method as claimed  
in any one of claims 16 to 21.

25     23. A kit for the detection of a target nucleic acid, comprising a labeled nucleic acid  
as claimed in claim 22.

24. A solid support to which is attached a reagent as claimed in any one of claims 1  
to 11.

30     25. A method for the capture of nucleic acids, comprising the following steps :

- providing a solid support to which is directly or indirectly attached at least one biological molecule as claimed in claim 15, or a nucleic acid as claimed in claim 22, the biological molecule or the nucleic acid comprising a diazomethyl function,
  - bringing into contact a biological sample which may contain free nucleic acids, and
- 5     • washing the solid support where the molecule(s) is (are) covalently attached at least to a nucleic acid.